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AIM2 Joins the Gang of Microbial Sensors

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AIM2 was recently implicated in the assembly and activation of the inflammasome in response to eukaryotic and viral dsDNA. Sauer and colleagues (2010) demonstrate that AIM2 is also involved in the activation of the inflammasome in response to the intracellular bacterium *Listeria monocytogenes*.

Mammalian cells are equipped with an array of germline-encoded receptors, called pattern-recognition receptors (PRRs), whose function is to detect conserved components of microbes, also known as pathogen-associated molecular patterns (PAMPs). Upon recognition of PAMPs, PRRs initiate host defense responses against invasive pathogens, thus promoting the removal of harmful microbes. Examples of PAMPs that are exclusive to microbes are lipopolysaccharide, a major component of the outer layer of Gram-negative bacteria and peptidoglycan, the main component of the cell wall of Gram-positive bacteria. Nucleic acids (i.e., DNA and RNA) represent another class of PAMPs. Although PRRs have evolved to discriminate between microbial versus endogenous nucleic acids, this ability is not absolute, as endogenous nucleic acids can also be released from cells and act as danger signals to trigger inflammation. However, the host has evolved several mechanisms to prevent endogenous nucleic acids from inducing an unnecessary inflammatory response (Takeuchi and Akira, 2010). Different classes of PRRs have been implicated in the recognition of DNA. TLR9 is localized in the endosome and recognizes unmethylated CpG DNA motifs, which are frequently present in microbes but rare in mammalian cells. The presence of double-stranded (ds) DNA in the cytosol is known to trigger immune responses (Takeuchi and Akira, 2010). DAI (DNA-dependent activator of IRFs) is localized in the cytosol and has been suggested to recognize dsDNA. However, DAI-deficient mice have an intact response to dsDNA, suggesting that the role of DAI in DNA sensing is redundant (Takeuchi and Akira, 2010). In some cell types, dsDNA can be transcribed into RNA via RNA polymerase III, which in turn activates the RNA sensor RIG-I (retinoid acid-inducible gene I). Activation of TLR9, DAI, or RIG-I leads to the transcriptional upregulation of proinflammatory cytokines and type I interferons (IFNs).

While the aforementioned PRRs induce transcriptional activation of inflammatory genes and type I IFNs in response to nucleic acids, a different receptor, AIM2 (absent in melanoma 2), is responsible for triggering the activation of the inflammasome in response to dsDNA. The inflammasome is a molecular platform that drives the activation of caspase-1, a cysteine protease responsible for the cleavage and maturation of the proinflammatory cytokines, IL-1β and IL-18 (Franchi et al., 2009). Several inflammasomes have been described to date, each of which is activated by different stimuli. For example, the NLRC4 inflammasome is activated by cytosolic flagellin, the mouse Nlrp1b inflammasome by anthrax lethal toxin, and the NLRP3 inflammasome by an array of stimuli that includes particulate/crystalline matter, bacterial pore-forming toxins, and ATP. NLRC4, Nlrp1b, and NLRP3 belong to the Nod-like receptor (NLR) family while AIM2 is a member of the Pyrin (PYD) and HIN200 domain-containing (PYHIN) family (Hornung and Latz, 2010). A feature shared between the inflammasome-activating NLRs and AIM2 is the use of the adaptor ASC (apoptosis-related speck-like protein), which links NLRs and AIM2 to procaspase-1. Early studies demonstrated that AIM2 binds dsDNA of both endogenous and microbial origin through its HIN200 domain, and upon activation, AIM2 oligomerizes and recruits the adaptor ASC through homophilic PYD interactions. In turn, ASC associates with pro-caspase-1 via CARD-CARD domain interactions, a critical step in inducing the activation of caspase-1.

The function of AIM2 in caspase-1 activation induced by particular microbes has remained unclear, although initial work suggested a role for AIM2 in the recognition of DNA viruses and *Francisella tularensis*, an intracellular bacterial pathogen (Hornung and Latz, 2010). A recent flurry of reports has provided clear evidence for a role of AIM2 in the host defense against several bacterial and viral pathogens. In this issue of *Cell Host & Microbe* (Sauer et al., 2010), Sauer and colleagues performed a forward genetic screen for *Listeria monocytogenes* mutants that exhibited altered ability to induce pyroptosis, a type of cell death in macrophages that relies on caspase-1 activation. They identified a *Listeria* mutant, *ΔImo2473*, with increased pyroptotic activity. Importantly, caspase-1 activation and IL-1β secretion induced by the *ΔImo2473* mutant was dependent on ASC, but independent of NLRC4 and NLRP3. Subsequent RNA interference experiments revealed that AIM2 was the crucial inflammasome engaged in macrophages infected with *Listeria ΔImo2473* mutants. Using *Listeria* strains engineered to express firefly luciferase, the authors found that not only *Listeria ΔImo2473* mutants but also wild-type bacteria treated with antibiotics preferentially triggered the activation of the AIM2 inflammasome. The activation of the AIM2 inflammasome infected with antibiotic-treated *Listeria* required the expression of listeriolysin O, suggesting that bacterial escape from the phagosome to the cytosol is necessary for the activation of

the AIM2 inflammasome. Future studies will be necessary to clarify the mechanism by which the *Imo2473* mutation increases bacterial susceptibility to lysis and AIM2 activation. The inflammasome involved in the activation of caspase-1 by *Listeria* has been controversial. Although it was first suggested that the NLRP3 inflammasome was crucial, subsequent studies found that NLRP3 was dispensable and suggested that a different inflammasome was engaged, depending on how *Listeria* was grown in culture. In a separate publication, Kim et al. (Kim et al., 2010) further clarified the role of AIM2 and NLRP3 in the activation of caspase-1 induced by *Listeria*. The authors showed that knocking down the expression of AIM2 in NLRP3-deficient macrophages greatly reduces the activation of caspase-1 in macrophages infected with *Listeria*. Collectively, these data suggest that the activation of caspase-1 triggered by *Listeria* infection proceeds through the engagement of different inflammasomes and additionally uncover a role for bacterial lysis in the activation of the AIM2 inflammasome.

Functional analysis using RNA interference is a powerful approach, but it can lead to artifacts due to off-target effects. In independent studies, two groups (Fernandes-Alnemri et al., 2010; Rathinam et al., 2010) generated AIM2-deficient mice using gene-trap technology. Consistent with previous reports, macrophages from AIM2-deficient mice showed an absolute requirement for AIM2 in caspase-1 activation induced by dsDNA. Notably, Fernandes-Alnemri et al. showed that the activation of the AIM2 inflammasome is critical for innate immunity to *Francisella* in that AIM2-deficient mice are significantly more susceptible to subcutaneous infection than wild-type mice (Fernandes-Alnemri et al., 2010). In their work, Rathinam et al. showed that AIM2 is important in caspase-1 activation triggered by two DNA viruses, vaccinia and mouse cytomegalovirus, and demonstrated that the AIM2 inflammasome is implicated in the early control of viral replication by promoting the production of IL-18 as well as IFN-γ produced by NK cells (Rathinam et al., 2010). Together, these studies highlight an important role for the AIM2 inflammasome in the innate immune response against specific intracellular pathogens and pave the way for future studies aimed to better define the role of AIM2 in innate and adaptive immune responses.

Another aspect that will require further investigation is the role of AIM2 in the recognition of endogenous DNA. Although TLRs preferentially recognize nucleic acids of microbial origin, recognition of endogenous nucleic acids by TLR can occur, resulting in exacerbated autoimmune disease. Unlike TLRs, AIM2 appears to recognize both self and nonself dsDNA with the same efficacy, and thus it may contribute to the pathogenesis of autoimmune disorders. In particular, it will be important to examine the role of AIM2 in autoimmune diseases characterized by abnormal accumulation of dsDNA. For example, DNase II-deficient mice accumulate undigested DNA in macrophages and develop severe arthritis (Okabe et al., 2005). Similarly, DNase I-deficient mice develop lupus-like disease (Napirei et al., 2000). This model may be clinically relevant, because some patients with heterozygous nonsense mutation in DNase I produce high levels of autoantibodies while patients with systemic lupus erythematosus can exhibit reduced DNase I activity in serum. Because cytosolic dsDNA also induces the production of type I IFNs, the contribution of AIM2 to disease remains unclear and needs to be addressed in future studies.

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